# Trends in Endocrinology and Metabolism Oxytocin, the sweet hormone?

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# Oxytocin, the sweet hormone?

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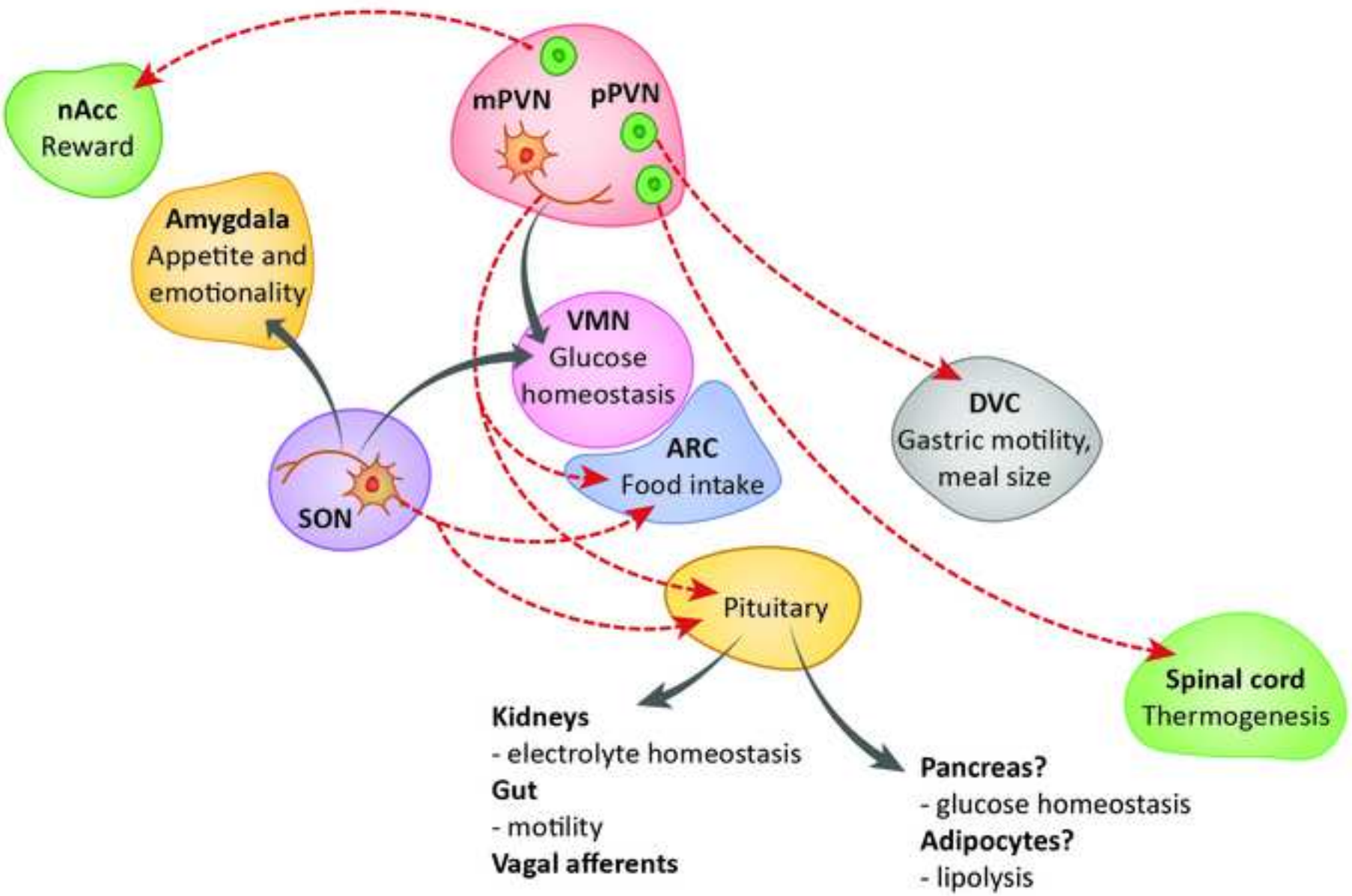
| **Abstract:** | Mammalian neurons that produce oxytocin and vasopressin apparently evolved from an ancient cell type with both sensory and neurosecretory properties that probably linked reproductive functions to energy status and feeding behavior. Oxytocin in modern mammals is an autocrine/paracrine regulator of cell function, a systemic hormone, a neuromodulator released from axon terminals within the brain, and a "neurohormone" that acts at receptors distant from its site of release. In the periphery, oxytocin is involved in electrolyte homeostasis, gastric motility, glucose homeostasis, adipogenesis and osteogenesis, and within the brain it is involved in food reward, food choice and satiety. Oxytocin preferentially suppresses intake of sweet-tasting carbohydrates while improving glucose tolerance and supporting bone remodelling, making it an enticing translational target. |

**Trends Box (713 characters)**

- In a wide range of species, from invertebrates to mammals, oxytocin-like neuropeptides link nutrient availability to feeding behavior and reproductive behaviors.
- In the brain, oxytocin signals not only to the classical appetite-regulating centres, but also to brain regions involved in food reward.
- Oxytocin preferentially suppresses the intake of sweet-tasting carbohydrates, both by actions on the brain's reward systems and by effects on sweet taste receptors.
- A major site of action is the ventromedial hypothalamus, which has a key role in glucose homeostasis as well as in reproductive behavior.
- In the periphery, intrinsic oxytocin systems in the gut regulate glucose homeostasis and gut motility.

Key Figure





# Oxytocin, the sweet hormone?

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Key words: hypothalamus, oxytocin, appetite, ventromedial nucleus, energy balance, gastrointestinal tract, satiety

**Abstract**

Mammalian neurons that produce oxytocin and vasopressin apparently evolved from an ancient cell type with both sensory and neurosecretory properties that probably linked reproductive functions to energy status and feeding behavior. Oxytocin in modern mammals is an autocrine/paracrine regulator of cell function, a systemic hormone, a neuromodulator released from axon terminals within the brain, and a "neurohormone" that acts at receptors distant from its site of release. In the periphery, oxytocin is involved in electrolyte homeostasis, gastric motility, glucose homeostasis, adipogenesis and osteogenesis, and within the brain it is involved in food reward, food choice and satiety. Oxytocin preferentially suppresses intake of sweet-tasting carbohydrates while improving glucose tolerance and supporting bone remodelling, making it an enticing translational target.

**Oxytocin: convergent roles of central and peripheral oxytocin in glucose homeostasis?**

The classical roles of oxytocin are to mediate the milk-ejection reflex and to regulate uterine contractility [1]. Since the discovery of centrally-projecting oxytocin neurons, it has become apparent that oxytocin also acts within the brain where it is important for many reproductive and social behaviors, including sexual behavior, maternal behavior and (in monogamous species) pair bonding [2-4]. However it has also become apparent that oxytocin is involved in some behaviors that are not obviously related to reproduction, and in particular, that it is involved in regulating food intake. Early experiments revealed an inverse relationship between pituitary oxytocin secretion and sodium appetite in rats [5], and as it emerged that oxytocin secretion promoted natriuresis [6], it was proposed that peripherally and centrally secreted oxytocin act in concert to stimulate sodium excretion while inhibiting sodium ingestion [5].

In recent years however, evidence has accumulated for a role of oxytocin in *food choice* (Fig. 1). Oxytocin neurons are preferentially activated by ingestion of sweet-tasting carbohydrates, while oxytocin preferentially inhibits their ingestion [7]. The central sites of actions of oxytocin include the ventromedial nucleus, an area prominently implicated in the central regulation of glucose homeostasis, while peripheral sites of oxytocin actions include the pancreas. Taste buds also express oxytocin receptors [8], and, at these, oxytocin may modulate processing of sweet taste, while central actions of oxytocin on reward pathways may modulate the processing of food reward.

**Oxytocin, an ancient peptide regulating metabolism and reproduction**

Oxytocin, at first sight, is a quintessentially *mammalian* hormone: its one assuredly indispensable role is to mediate milk let-down in response to suckling during lactation [9]. In all mammals, either oxytocin or mesotocin is produced by hypothalamic neurons, many of which project to the neurohypophysis, from where it is secreted into the systemic circulation. Mesotocin, which is present in marsupials, differs from oxytocin by one amino acid in a neutral mutation, but binds equivalently at oxytocin receptors [10]. All mammals also have a second neurohypophysial hormone, vasopressin, which is so closely related to oxytocin that it clearly arose from a gene duplication event in evolution [11]. But it is not only mammals that have a neurohypophysis – all vertebrates do, and most have both an oxytocin-like peptide and a vasopressin-like peptide, so the gene duplication must have occurred early in chordate history.

In invertebrates, oxytocin/vasopressin-like peptides emerged at about the same time as bilateral symmetry in body plans, and they are found in many modern nematodes, insects, annelids and molluscs [12]. In annelids and zebrafish, the neurons that produce the oxytocin homolog express common tissue-restricted microRNAs and a common cell-type-specific combination of transcription factors, indicating that they evolved from an ancient cell type with both sensory and neurosecretory properties [12]**.** These invertebrate peptides are consistently implicated in the regulation of reproductive behaviors, and, in many species, reproduction depends on food availability and is regulated by metabolic cues. For example, in *C elegans*, the oxytocin-like peptide nematocin modulates both male mating circuits and a gustatory plasticity circuit that directs food preference. Nematocin acts at two G protein-coupled receptors, NTR-1 and NTR-2, and most of the cells that express these receptors have no synaptic connections to any nematocin-expressing cells [13, 14]. Thus, in *C elegans*, nematocin communicates with its target cells not as a "conventional" neurotransmitter, but as a local paracrine or hormone-like messenger [13], as oxytocin does in the mammalian brain.

**Oxytocin and the control of appetite**

In rats, central (intracerebroventricular) injections of oxytocin affect social and sexual behavior, but also potently inhibit appetite [15-17], as do central injections of mesotocin in chicks [18]. In mammals, oxytocin is expressed in magnocellular neuroendocrine neurons that project to the neurohypophysis and in parvocellular neurons of the paraventricular nucleus (PVN), subsets of which project to forebrain regions, the caudal brainstem and spinal cord. Exactly how many anatomically distinct populations of parvocellular oxytocin neurons there are is not known, nor is it known how functionally heterogeneous these populations are, or how disparate are their responses to different stimuli (Box 1).

Oxytocin projections from parvocellular neurons of the PVN densely innervate neurons of the dorsal vagal complex, particularly in the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus, where there is a high density of oxytocin receptors. Other projrctions enter the spinal cord, with effects on thermogenesis as well as on pain processing [19] and erectile function [20]. The digestive functions of the stomach and esophagus are co-ordinated by parasympathetic and sympathetic reflexes that are regulated directly or indirectly by the PVN. In turn, NTS neurons project back to the PVN and other areas of the hypothalamus to control feeding behavior, and are important for meal termination. Thus modulation of the dorsal vagal complex by the PVN, including by oxytocin neurons, controls both gastric motility and feeding behavior (Box 2) [21, 22].

Oxytocin is clearly *not* essential for the control of feeding. Oxytocin knock-out mice eat similar amounts as wild-type mice in basal conditions; they are prone to late-onset obesity, but this seems to reflect a decreased sympathetic tone rather than hyperphagia [23]. However, they show a greater preference for palatable sucrose or saccharin solutions [24] and this change appears to be selective, as oxytocin knock-out mice do not over-consume palatable fat-containing solutions [25]. Several lines of evidence suggest that oxytocin may be specifically involved in regulating the intake of sweet-tasting carbohydrates**,** and this has been linked to actions of oxytocin in the nucleus accumbens, an important part of the brain's reward circuitry [26, 27]. Oxytocin here and other parts of the reward system including the ventral tegmentum [28] has also been implicated in reward associated with social interactions [29-31]. It has accordingly been suggested that oxytocin suppresses reward-driven food intake while enhancing social reward, and that it is a "conditional anorexigen", whose effects depend on physiological and social context [7].

Genetically targeted ablation of oxytocin neurons in adult mice does not affect food intake, body weight or energy expenditure in mice maintained on a normal diet [32] but, as a consequence of reduced energy expenditure, male mice lacking oxytocin neurons are more prone to obesity when they are fed a high-fat diet. Male mice lacking oxytocin neurons also show a blunted anorexic response to leptin but a normal response to a melanocortin agonist [32], which is also surprising given that leptin is thought to mainly act on the PVN via melanocortin signaling. It seems that oxytocin neurons may be important for resisting diet-induced obesity, but their role in feeding is permissive and can be compensated for by other pathways.

**The magnocellular oxytocin system**

The *magnocellular* oxytocin neurons (Box 3), which are critically involved in the milk-ejection reflex and parturition [1], are also involved in regulating energy balance and gastric function [33]. The PVN contains magnocellular oxytocin neurons as well as parvocellular (centrally projecting) oxytocin neurons, together with an array of other neuronal populations involved in metabolic regulation, but the rat supraoptic nucleus (SON) contains only magnocellular oxytocin and vasopressin neurons, all of which project to the neurohypophysis. Both the oxytocin neurons and the vasopressin neurons of the SON are activated after feeding in rats and mice [34-36], and oxytocin secretion into plasma is increased during re-feeding after a fast. In time restricted-fed rats, the onset of feeding is

accompanied by prompt induction of expression of c-*fos* at many brain sites, including the NTS, and in many hypothalamic nuclei, but nowhere more strongly than in the SON [34]. The activation of magnocellular vasopressin neurons has received less attention, probably because it is assumed that this reflects an antidiuretic reflex in anticipation of the solute load that accompanies food intake [37]. Oxytocin released from the neurohypophysis is involved in electrolyte homeostasis: in rodents, it promotes natriuresis by stimulating natriuretic hormone secretion from the heart and by direct actions at the kidney.

The SON receives a strong projection from noradrenergic and peptidergic neurons in the NTS, and these preferentially innervate oxytocin neurons. In rats, systemic injections of cholecystokinin (CCK), a peptide hormone released from the duodenum in response to food ingestion, inhibit magnocellular vasopressin neurons but activate the oxytocin neurons [38] and stimulate oxytocin secretion into the blood. This response depends on activation of CCK1 receptors on the sensory endings of afferent neurons of the gastric vagus, and subsequent activation of noradrenergic (A2) neurons in the NTS [38], and of a subpopulation of NTS neurons that express prolactin-releasing peptide (PrRP). PrRP mediates, at least in part, the activation of oxytocin neurons in response to food intake, and the CCK-PrRP-oxytocin pathway is involved in the control of meal termination [36]. Gastric distension also activates magnocellular oxytocin neurons, probably also via activation of NTS neurons**,** though whether these are the same neurons as are activated by CCK is not known [39]. Both oxytocin and vasopressin neurons in the SON are also activated by systemic administration of secretin [40], a hormone secreted from the duodenum in response to food intake which regulates gastric secretion and emptying, and this again is mediated by a vagal projection to the NTS [41].

Glucagon-like peptide 1 (GLP-1), a hormone secreted the intestinal epithelium, is involved in both glucose homeostasis by its stimulatory effects on insulin secretion from the pancreas, and in appetite control. It is also expressed in gustatory neurons, and has been implicated in sweet taste transmission from taste buds [42]. Sweet taste receptors are also present in the gut and pancreas, and are important in glucose homeostasis during diet-induced obesity [43]. How important these peripheral taste receptors are for gut signaling to the brain remains to be established. The immediate-early gene c-*fos* has been extensively used as a marker of neuronal activation, as it is transiently expressed in many neurons following activation. Peripheral administration of GLP-1 induces expression of c-*fos* in the NTS and the SON but not in the PVN [44], while peripheral administration of the GLP-1 agonist exendin-

4 activates c-*fos* mRNA expression in the arcuate nucleus and parvocellular PVN at low doses, and at higher doses also activates the magnocellular PVN and the SON, effects mediated in part by the vagus [45]. GLP-1 is also expressed in some NTS neurons that project to the PVN and SON, and central administration of GLP-1 increases plasma oxytocin concentration in rats, and suppresses feeding [46].

The expression of oxytocin mRNA in the PVN and SON is suppressed by fasting in mice, and this is rescued by leptin administration [47]; similarly, in rats, fasting reduces the electrical activity of magnocellular oxytocin neurons, while systemically administered leptin enhances their electrical activity [48]. The magnocellular oxytocin neurons interact with the leptin-responsive pro-opiomelanocortin (POMC) neurons of the arcuate nucleus that produce the potent satiety peptide α-melanocyte stimulating hormone (α-MSH) together with a second anorectic neuropeptide, cocaine-and amphetamine regulated transcript (CART). The POMC neurons innervate both the PVN and the SON, which densely express MC3 and MC4 receptors through which α-MSH acts in the brain [49]. In SON oxytocin neurons, α-MSH induces mobilisation of intracellular calcium stores, expression of c-*fos*, and dendritic oxytocin secretion, but it also inhibits their electrical activity and therefore inhibits secretion from the neurohypophysis [49]. The inhibition of electrical activity is the consequence of evoked production of endocannabinoids, which act on afferent inputs to the SON, suppressing the release of excitatory transmitters from afferent endings that express cannabinoid receptors. Interestingly, in pregnant rats α-MSH has no effect on oxytocin neurons [50], and while icv α-MSH increases c-fos expression in the PVN, SON, arcuate nucleus, and ventromedial nucleus of the hypothalamus (VMN) in non-pregnant rats, this response is also suppressed in pregnant rats [50]. How this change arises is not known, as the hypothalamic levels of mRNA expression of MC3 and MC4 receptors are unchanged in pregnancy, but it may contribute to the hyperphagia of late pregnancy.

**Central targets of the magnocellular oxytocin system**

The magnocellular oxytocin system is the source of oxytocin in the plasma, but oxytocin is also released in abundance from the large *dendrites* of magnocellular neurons. Dendritic oxytocin release can be evoked by intracellular calcium mobilisation following activation of some G-protein coupled receptors, including those for α-MSH and for oxytocin itself. By contrast, release in response to electrical (spiking) activity depends on voltage-gated calcium entry and hence on the anatomical disposition of the vesicles in which oxytocin

is stored: to be available for such activity-dependent release, the vesicles must be located close to clusters of voltage-dependent calcium channels in the plasma membrane [51]. Normally, vesicles in dendrites are distanced from these sites by a network of filamentous actin, and can be released only by increases in intracellular calcium that open channels in this network [52]. However, activation of some peptide receptors can "prime" the dendritic pool of vesicles by promoting their relocation to juxta-membrane sites. Thus activity-dependent oxytocin release requires a prior priming event, and because priming takes many minutes, it results in a delayed but long-lasting activation of release [53].

So much oxytocin can be released from dendrites that it is in principle able to increase oxytocin concentrations substantially over large areas of the forebrain. Thus oxytocin signaling in many areas of the brain, like that of the ancestors of oxytocin cells in invertebrates, depends not on anatomical connectivity but only on the distribution of receptors [54]. The exact anatomical distribution of oxytocin released from dendrites is not known, but a substantial amount of oxytocin reaches the ventricles, as concentrations in CSF are much higher than in plasma. The half-life of oxytocin in CSF is about 20 min, but in neural tissue it is much shorter. Within neural tissue, oxytocin is degraded by a membrane-bound enzyme, placental leucine aminopeptidase (P-LAP) [55]. Thus exactly where dendritically-released oxytocin goes and in what quantities depends on the direction and speed of flow of oxytocin in extracellular fluid, and on the rate of degradation and binding along those paths, and these are ill-defined.

Dendritically-released oxytocin acts within the PVN and SON, including on the oxytocin neurons, but it is also likely to be the main source of oxytocin signaling to two relatively close sites that express abundant oxytocin receptors: the amygdala, which contains only a few oxytocin-containing fibres, and the VMN, which appears to contain none. The VMN is important for glucose homeostasis [56, 57], and controls sexual behavior, feeding, fear behavior and aggression, all of which are modulated by oxytocin at this site [58]. These behaviors are not mutually compatible: given the motivation and opportunity to have sex and to eat, animals generally do one or the other, unless they are afraid, in which case they may fight or flee but are unlikely to eat or mate. Oxytocin enhances sexual behavior while suppressing both feeding and fear, and so, by its actions at the VMN, oxytocin may be key to behavioral decisions. Magnocellular oxytocin neurons in the rat are rapidly activated by food intake, but dendritic release is delayed and long-lasting, potentially contributing to post-prandial satiety and promoting post-prandial sexual appetite [59].

Oxytocin also acts on the arcuate nucleus, and in this case the actions may be mediated by collaterals of the axons of magnocellular oxytocin neurons en route to the neurohypophysis. POMC cells express oxytocin receptors and are apparently in contact with oxytocin-containing axonal boutons [60]. Dopamine neurons in the arcuate nucleus also respond to oxytocin; these are neuroendocrine neurons that control prolactin secretion, but they have extensive intrahypothalamic projections and are integrated into the appetite-regulating circuitry [61]. Finally, the mouse arcuate nucleus contains glutamatergic neurons that project to the PVN and which express oxytocin receptors. Optogenetic or pharmacogenetic activation of these neurons rapidly inhibits feeding in mice, and the effect of oxytocin on these neurons is potentiated by α-MSH *in vitro* [62].

**Peripheral targets of meal-induced oxytocin release**

In rodents, oxytocin secretion promotes sodium excretion (natriuresis) by direct actions on oxytocin receptors at the kidney, and indirectly, by stimulating natriuretic hormone secretion from the heart, and it influences gastric motility [22, 63, 64]. However, these effects are not seen in all mammals. In man, oxytocin secretion is stimulated by high intensity exercise, and this is associated with altered fluid balance [65], but oxytocin secretion is *not* activated by osmotic stimuli as it is in rodents, nor does oxytocin induce natriuresis [66]. Oxytocin secretion in man is generally not stimulated by feeding-induced gastric distension or by systemic administration of CCK; instead, these stimuli activate *vasopressin* secretion, whereas CCK inhibits vasopressin secretion in rats.

However, oxytocin as well as its receptor is present throughout the gastrointestinal tract in human, guinea pig, rabbit and rat [67]. In mice, some enteric neurons and enterocytes express both oxytocin and oxytocin receptors, and oxytocin signaling not only acts as a brake on intestinal motility, but also decreases mucosal activation of enteric neurons, promotes enteric neuronal development and survival, regulates proliferation of crypt cells and mucosal permeability [68], and is protective against inflammation [69]. Oxytocin receptors are also expressed in the rat pancreas [70], and signaling through these stimulates insulin and glucagon secretion.

Adipocytes also express oxytocin receptors, and signaling though these induces lipolysis [71]. Adipocytes and osteoblasts arise from the same progenitor cells (marrow stromal cells). Osteoblasts produce oxytocin as well as oxytocin receptors, and there is evidence that oxytocin is a paracrine-autocrine regulator of bone formation that is modulated

by estrogens [72, 73]. Oxytocin increases osteoclast formation, and in mature osteoclasts it inhibits bone resorption. Mice lacking oxytocin or its receptor develop osteoporosis that worsens with age: their skeletons have a lower vertebral and femoral trabecular volume, osteoblasts exhibit less mineralization activity, and genes for osteoblast differentiation are down-regulated [74]. There is a reciprocal relationship between osteoblast and adipocyte differentiation: while glucocorticoids favor differentiation towards cells of the adipocyte lineage, oxytocin promotes osteogenesis in both human multipotent adipose-derived stem cells, and marrow stromal cells [75]. In rabbits, glucocorticoid-induced osteoporeosis can be prevented by systemic oxytocin administration [71], and in mice, oxytocin can reverse ovariectomy-induced osteopenia and adiposity [76].

Several studies in man and other animals have reported that systemically administered oxytocin affects appetite, weight gain, glucose homeostasis, lipid metabolism and thermoregulation. Many of these have involved doses of oxytocin that far exceed physiological circulating concentrations, doses that may result in actions at peripheral vasopressin receptors as well as oxytocin receptors [77], and at receptors that normally are regulated by local paracrine sources of oxytocin rather than blood-borne oxytocin of neurohypophysial origin. These doses are in many cases so high that, despite an effective blood-brain barrier to oxytocin, they may be active within the brain.

In leptin-deficient mice and in leptin-resistant mice [16], systemic administration of oxytocin at doses of 5-150 μg/day reduces food intake and adiposity, but worsens glucose homeostasis, possibly by stimulating corticosterone production. These daily doses exceed the total pituitary content of oxytocin by at least 10 fold, and at these high doses some oxytocin is likely to enter the brain despite the presence of a very effective blood-brain barrier to oxytocin. However, their effects still may be exerted at peripheral locations, as the effects on feeding involve actions of oxytocin on the nerve endings of afferent vagal neurons [78]. In rhesus monkeys, systemic administration of comparably large doses of oxytocin (200-400 μg/kg) reduce body weight and increases energy expenditure and lipolysis [79], again possibly by peripheral actions. In rats, peripheral injections of oxytocin inhibit sucrose intake and sucrose-seeking behaviour, but only at extremely high doses (3 mg/kg i.p.) [80].

In man, intravenous infusions of oxytocin at doses in the physiological range have little effect on food intake or gastric emptying in normal subjects, but a number of recent studies have looked at the effects of intranasal application of oxytocin on food intake in man. These involve very high doses of oxytocin that raise plasma concentrations to

supraphysiological levels; a small amount of the applied oxytocin probably reaches the brain, but whether it does so in effective amounts is uncertain [81]. These studies typically show reductions in food intake, and Ott et al. [82] reported a marked reduction in intake of sweet snacks after intranasal administration of 24 IU (~50μg). This does not necessarily imply a central action, as in humans and in mice, taste buds express oxytocin receptors [8], and in mice oxytocin decreases sweet taste sensitivity [83]. Intranasal oxytocin has also been reported to improve glucose tolerance by effects on pancreatic beta cells [84]. Given the diversity of peripheral tissues that express oxytocin receptors, and that intranasally administered oxytocin may also activate vasopressin receptors at many peripheral sites, it remains to be established what if any of the claimed effects of intranasal oxytocin actually involve actions in the brain.

## Concluding remarks

Oxytocin has multiple roles in energy balance and metabolism, some of which are exerted in the CNS, some peripherally by secretion from the neurohypophysis, and some by local paracrine actions of an oxytocin system intrinsic to the gut. Oxytocin inhibits food intake, and preferentially inhibits the intake of sweet-tasting carbohydrates, in part by actions on the appetite regulating centres of the hypothalamus, in part by actions at sweet taste receptors, and possibly also by actions on the brain's reward centres. It increases energy expenditure and lipolysis, slows gastric motility, and improves glucose tolerance by actions at the pancreas. These actions have their evolutionary origin in an ancient cell type with both sensory and neurosecretory properties that probably linked reproductive functions to energy status and feeding behavior. Particular translational interest lies in the effects of oxytocin on bone remodelling and on effects on food preference and energy expenditure; difficulties in developing therapeutic applications will mainly arise from preventing off-target effects and their unintended consequences, particularly of long-term treatment. Mechanistic understanding remains incomplete (see *Outstanding Questions*), and a current problem in distinguishing physiological from pharmacological effects of systemic oxytocin arises from pervasive confusion about measurements of oxytocin in plasma [85].

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**Box 1: The oxytocin systems of the mammalian brain**

The distribution of oxytocin neurons in the brain is relatively consistent across species, but the distribution of projections is more variable and that of receptors is very variable. The main aggregations in rats are in the PVN, extending into the adjacent periventricular nucleus, and in the SON. A third, rostral aggregation, sometimes called the anterior commisural nucleus, extends into the bed nucleus of the stria terminalis and the preoptic area. However, many neurons are scattered as isolated cells or small clusters in the lateral and anterior hypothalamic areas between the PVN and the SON, and some appear in the dorsomedial hypothalamic nucleus. In the mouse, a few oxytocin cells surpass the rostral border of the SON, impinging into the medial preoptic area, and the medial amygdala [86].

In the rat, oxytocin neurons project to all main regions of the forebrain [87], as well as to the dorsal vagal complex in the caudal brainstem and to the spinal cord. The anatomical distribution of oxytocin neurons and fibres is relatively consistent amongst mammals, but there is considerable species variation in oxytocin receptor expression, reflecting differences in the oxytocin receptor gene [88], and this is associated with differences in reproductive behavior. For example, in rodents oxytocin receptors are concentrated in brain regions involved in olfactory processing, but in primates they are concentrated in regions involved in visual processing and attention [89].

Species differences in receptor expression are not matched by equivalent differences in the anatomy of oxytocin pathways, suggesting that oxytocin signaling extensively reflects extrasynaptic release of oxytocin. In the rat, the olfactory bulb, ventral pallidum, medial

preoptic area, and VMN all express moderate to high levels of oxytocin receptors but are not directly innervated by oxytocin neurons. The last three of these regions are close to the PVN and SON, and are thus likely to be reached by dendritically released oxytocin; the olfactory bulb seems likely to receive oxytocin via the CSF. Of these sites, the VMN appears to be the most evolutionarily conserved region of receptor expression. Oxytocin receptor binding is prominent in the VMN in mice, rats, guinea pigs, prairie and montane voles, and in rhesus monkeys [90] and humans [91].

**Box 2: The PVN and the dorsal vagal complex.**

The gastrointestinal tract has an intrinsic nervous plexus that gives the intestine considerable neural autonomy, but the digestive functions of the stomach and esophagus are co-ordinated by parasympathetic and sympathetic reflexes which are regulated by the dorsal vagal complex, comprising the NTS, the dorsal motor nucleus of the vagus and a circumventricular organ, the area postrema, which are densely interconnected. This complex is regulated directly and indirectly by the PVN.

Gastric distension and gastric hormones stimulate sensory vagal afferent pathways which activate NTS neurons, including noradrenergic neurons of the A2 cell group and other neurons that express a variety of neuropeptides, while blood-borne gastric hormones activate the area postrema, which lies outside the blood-brain barrier. A projection from the NTS to the dorsal motor nucleus of the vagus regulates efferent vagal activity, completing a vago-vagal reflex loop. This reflex is modulated by a recurrent neural circuit from the NTS to the PVN, and from the PVN back to the dorsal vagal complex, and this involves a projection from parvocellular oxytocin neurons. The NTS neurons also project to the hypothalamus to control meal termination.

The PVN is characterised by expression of the transcription factor *Sim1*, and ablation of Sim1 neurons in mice results in hyperphagia and altered energy expenditure [92, 93]. Conversely, activation of Sim1 neurons in the PVN suppresses feeding, and this is mediated by Sim1 neurons that express nitric oxide synthase. These include both magnocellular and parvocellular oxytocin neurons, but in mice, pharmacogenetic activation of the PVN oxytocin neurons alone is not sufficient to suppress food intake [94].

However, some parvocellular oxytocin neurons project to the spinal cord, and direct activation of PVN Sim1 neurons increases energy expenditure and increases intrascapular temperature overlying brown adipose tissue, and PVN oxytocin neurons that project to the spinal cord contribute to this response [94].

The arcuate nucleus neurons that co-express neuropeptide Y and agouti-related peptide (AgRP) are obligatory for feeding behavior at least under normal circumstances. These neurons target and inhibit oxytocin neurons in the PVN [95], and the feeding that is evoked by activation of the AgRP neurons involves suppression of oxytocin neuron activity. This does not imply that the PVN oxytocin neurons are *essential* for the control of feeding: different subsets of AgRP neurons project to different targets which can independently activate feeding [96].

**Box 3: The magnocellular oxytocin neurons**

In the rat, the magnocellular oxytocin neurons comprise about 10,000 neurons in the SON, PVN, and in scattered cells and smaller aggregations (accessory nuclei) between these main aggregations. These neurons all project a single axon to the neurohypophysis from where they secrete oxytocin into the systemic circulation, but some also project centrally, including to the amygdala and to the arcuate nucleus. The neurons each have 1-3 thick dendritic processes which contain abundant oxytocin-containing vesicles, and are a major source of oxytocin secretion within the hypothalamus. In the SON, these dendrites form a dense mat at the base of the brain (**Figure 2**). Oxytocin secretion from dendrites can be triggered by peptides which mobilise intracellular calcium from intracellular stores without any increase in electrical spike activity, and hence independently of secretion from their axonal nerve endings in the neurohypophysis. Dendritic secretion can also be evoked by spike activity, but this requires prior "priming" of the dendritic pool of vesicles.

The magnocellular oxytocin and vasopressin neurons appear to act as glucose and metabolic sensors [97]. The oxytocin neurons co-express several other anorectic peptides, including CCK and nesfatin, which is also expressed in parvocellular oxytocin neurons [98] and in many magnocellular vasopressin neurons [99], and express receptors for many appetite-regulating peptides, including the peripherally produced hormones insulin and leptin and the centrally produced anorectic peptides α-MSH and CCK. They produce endocannabinoids and nitric oxide in an activity-dependent manner, and they also express the vesicle glutamate transporter VGLUT2, so their central projections appear to use glutamate as a conventional neurotransmitter. The promoter region of the oxytocin gene contains a putative transcription factor binding site for retinoid-related orphan receptor α, which is activated by the metabolic regulator, peroxisome proliferator-activated receptor coactivator-1α (PGC-1α) [100].

**Key Figure: Central and peripheral targets of the brain oxytocin systems in the regulation of energy balance and metabolism.**

Oxytocin is expressed by neurons of the PVN and SON. Parvocellular oxytocin neurons in the PVN (pPVN) project axons to diverse sites (as indicated by the dotted lines), including to the amygdala, nucleus accumbens (nAcc), dorsal vagal complex (DVC) and spinal cord, each of which contains oxytocin receptor-expressing neurons and is importantly involved in the regulation of energy balance. Magnocellular oxytocin neurons in the SON and mPVN project to the posterior pituitary, from where oxytocin is secreted into the systemic circulation. Effects of circulating oxytocin on gut motility and (in rats) on sodium excretion from the kidneys are well established, but the pancreas and adipocytes also express oxytocin receptors and may be targets of circulating oxytocin. The arcuate nucleus (ARC) also receives afferent oxytocin fibers, shown here as coming from magnocellular neurons, but possibly coming from parvocellular neurons. Oxytocin receptors are very densely expressed in the ventromedial nucleus (VMN) and amygdala: these sites contain very few oxytocin fibres but are likely to be accessed by extrasynaptic oxytocin release, including oxytocin secreted from the dendrites of magnocellular neurons in the SON and PVN (as indicated by the black arrows).

**Figure 2: The rat SON**

In the rat, magnocellular oxytocin neurons (red) mainly occupy the dorsal SON while vasopressin neurons (green) predominate in the ventral SON. The dendrites of the oxytocin neurons form a dense mat at the base of the nucleus. Image by courtesy of Mike Ludwig.

Figure 2

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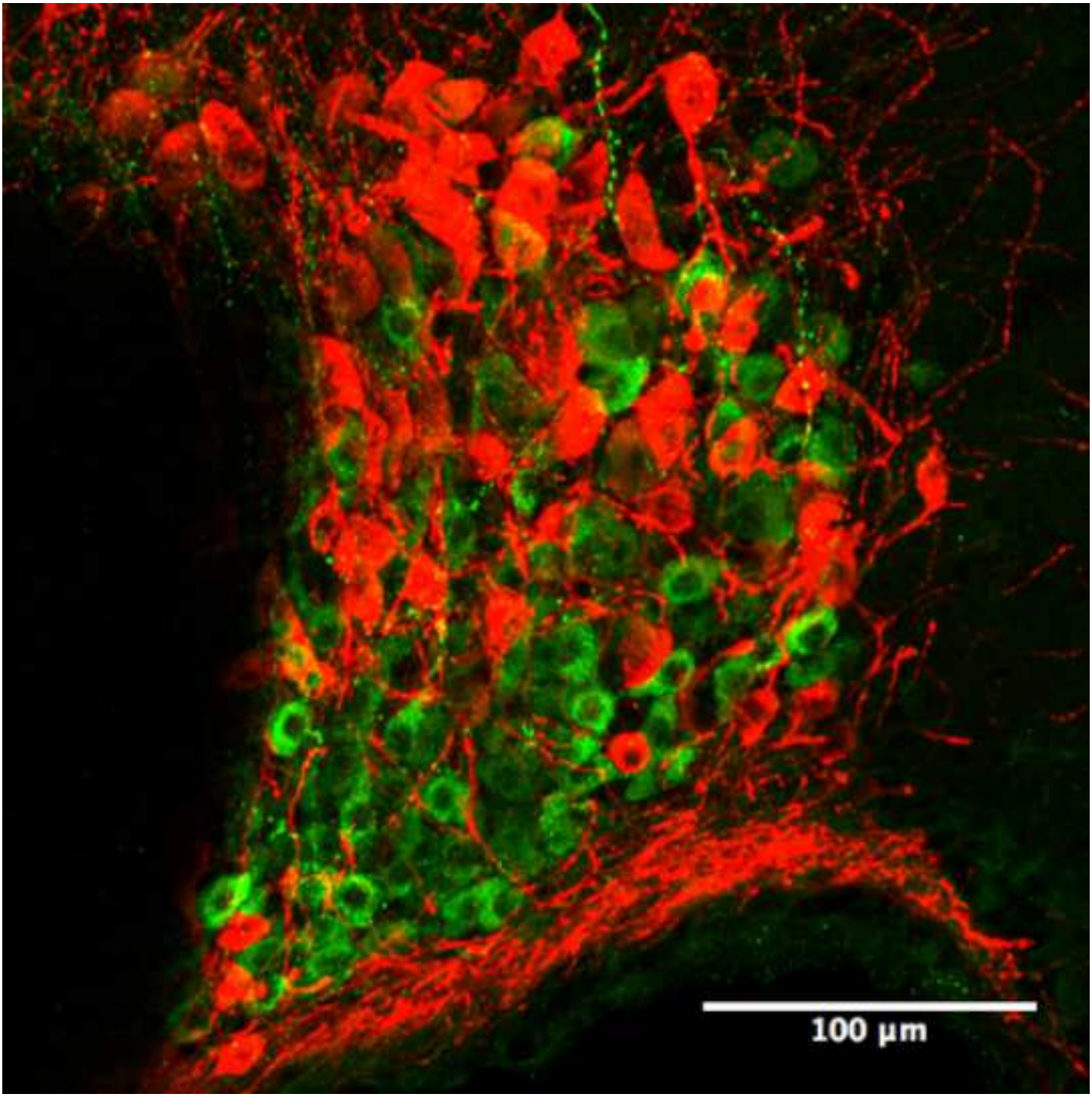

**Outstanding Questions Box (1232 characters)**

- How independent are the central oxytocin systems – are there many functionally distinct populations, or is it reasonable to think of the oxytocin systems of the brain as a functionally coherent system?
- Are magnocellular oxytocin neurons preferentially activated by ingestion of sweet-tasting carbohydrates?
- Sweet receptors are present in the mouth and also in the gut – how does signaling from these affect the oxytocin systems?
- By which pathways does the ventromedial nucleus regulate glucose homeostasis: does this involve efferent regulation of insulin and glucagon secretion?
- Does circulating oxytocin regulate the oxytocin receptors in the gut, or are these regulated solely by locally-produced oxytocin?
- In pregnancy and lactation, the oxytocin system undergoes extensive adaptations – how do these affect food choice and glucose homeostasis?
- The central oxytocin systems also regulate social and sexual behavior – how exactly are these linked to food choice?
- The spinally projecting oxytocin neurons have been implicated in pain processing, thermogenesis and penile erectile functions – what, if anything, links these apparently diverse actions?
- Is the gut oxytocin system autonomous, or is it regulated by the CNS?

Response to reviewers:

Dear Editors,

We thank the editor and reviewers for their kind comments and very helpful constructive suggestions.

The reviewer raised one major issue, with which the editor agreed – that the main figure was disappointingly simplistic.

We have taken this comment very seriously, and constructed a wholly new version with the aid of Elsevier Illustration Services. We hope that this is now more appropriate as a Key Figure.

The referee made additional very helpful and constructive comments that we have paid careful note of in the revision, and we also thank the editor for the helpful mark-up and comments.

We have added 20 references, bringing the total to 100; the additional references meet specific points that the editor felt needed references, while also referencing the introductory paragraphs more fully as requested by the reviewer, and adding references to the reward section.

The text is modestly extended, mainly by inclusion of an introductory paragraph on the “classic” roles of oxytocin as suggested by the referee.

We hope that the revised version is now acceptable.

Gareth Leng